Chapter 1
Earthworm Morphology

Earthworms are bilaterally symmetrical, externally segmented, with a corresponding internal segmentation. They have no skeleton and a thinly pigmented cuticle bearing setae on all segments except the first two, with an outer layer of circular muscles and an inner layer of longitudinal muscles. Earthworms are hermaphrodite and have relatively few gonads, situated in consistent segmental positions. When mature, and after fertilization of the eggs, a swollen area of the epidermis termed a clitellum, located in particular segments, forms a cocoon in which the eggs or ova are deposited, and this is then passed over the anterior segment. The eggs are usually fertilized as the clitellum passes along the body and is extruded over the head as a cocoon and the young develop within the eggs without a pre-larval stage, the newly hatched worms resembling adults. A few species can reproduce occasionally or consistently parthenogenetically.

Structurally, earthworms have large coelomic cavities containing coelomocytes, a closed vascular system with at least a dorsal and a ventral trunk and a ventral nerve cord. The alimentary canal is basically an anterior–posterior tube with excretion through the anus or specialized organs called nephridia; respiration is mainly cuticular. These systems were discussed further by Wallwork (1983).

1.1 Segmentation

Earthworms are divided externally into bands or segments along the length of the body by furrows or intersegmental grooves, which coincide with the positions of the septa dividing the body internally. The segments vary in width, usually being widest in the anterior and clitellar regions of the body. Segments are numbered arbitrarily from the front to the rear, and the grooves are designated by the numbers of the segments on either side, e.g., 3/4, 10/11, etc. Often the external segments are

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Plate 1.1 Prostomium of *Aporrectodea caliginosa*

subdivided by one or two secondary grooves, particularly in the anterior part, but these are superficial divisions which are not reflected in the internal anatomy. The mouth opens on the first segment, or peristomium, which bears on its dorsal surface the prostomium, a lobe overhanging the mouth (Plate 1.1). The prostomium varies in size, and in some worms, it may be so small that it cannot be distinguished. The way in which the peristomium and prostomium are joined differs between species and is a useful systemic character. The connection is termed zygolobous, prolobous, epilobous, or tanylobous depending on the demarcation of the prostomium (Fig. 1.1). The morphology of the segmentation of earthworms can change in response to soil contaminants such as antimony (Sb), becoming "broken" in appearance (Huang et al., 2021; Xu et al., 2021). More information regarding the effects of chemicals on earthworms can be found in Chap. 13, effects of agricultural practices and chemicals on earthworms.

1.2 Chaetotaxy

The setae are bristle-like structures borne in follicles on the exterior of the body wall which can be extended or retracted by means of protractor and retractor muscles that are attached to the base of the follicles and pass through the longitudinal muscle layer into the circular muscle layer below. As the setae are used to grip the substrate,

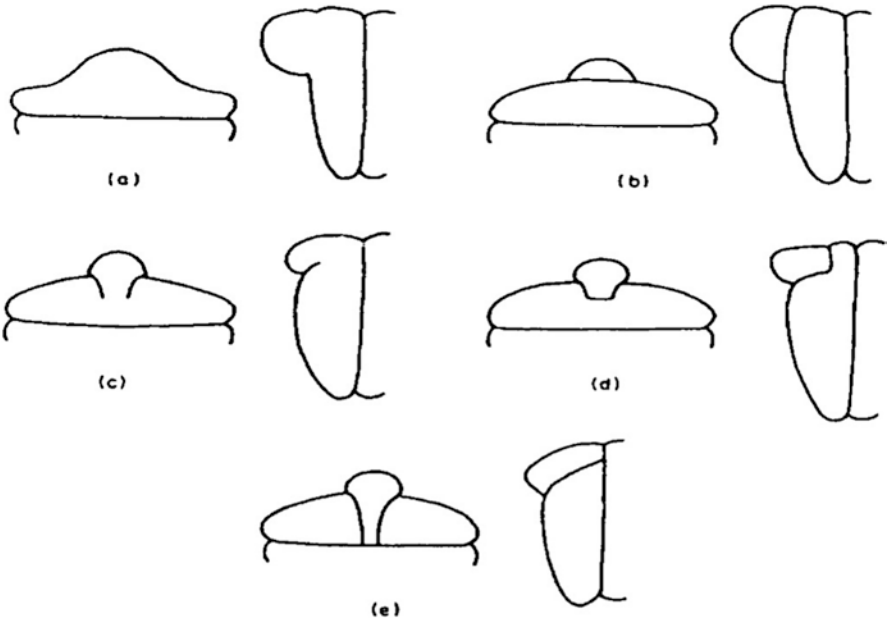

Fig. 1.1 Various forms of prostomium (cephalization). (**a**) Zygolobous; (**b**) prolobous; (**c, d**) epilobous; and (**e**) tanylobous. Some species have unusual segmentation in the region just before the anus. For instance, *Pontoscolex* (Glossoscolecidae) has a swollen posterior end, with narrow segments; it has been suggested this is a tactile sensory region adapted for gripping the sides of the burrow to avoid being pulled from the ground

their principal function is locomotory. Different species of oligochaetes have setae of varying shapes – either rod-, needle- or hair-like. Rarely, the distal end of a seta is forked. The shape of setae varies with their position, the most common form being those of *Lumbricus*, which are sigmoid and about 1 mm long. Some setae are enlarged both at the anterior and posterior ends, as in *L. terrestris*; the setae in the region of the genital pores (particularly the male pores) are sometimes modified in size and shape and situated on raised papillae. The genital setae, which can be up to 7 mm long in some species of Lumbricidae, are usually grooved along their length and may have a hook-like process at the distal end. These setae assist the physiological processes which take place at copulation by providing physical stimuli to the partner, while other setae have been shown to assist in holding copulating earthworms together by gripping, clasping, or penetrating the skin.

The setae are arranged in a single ring around the periphery of each segment, their number, and distribution being termed either lumbricine or perichaetine. The lumbricine arrangement, typical of the Lumbricidae, consists of eight setae per segment in ventral and latero-ventral pairs. If the distance between the setae in each pair is very small, they are termed "closely paired," if wider apart, they are termed "widely paired," or if they are very far apart so that the pairing is not obvious, they

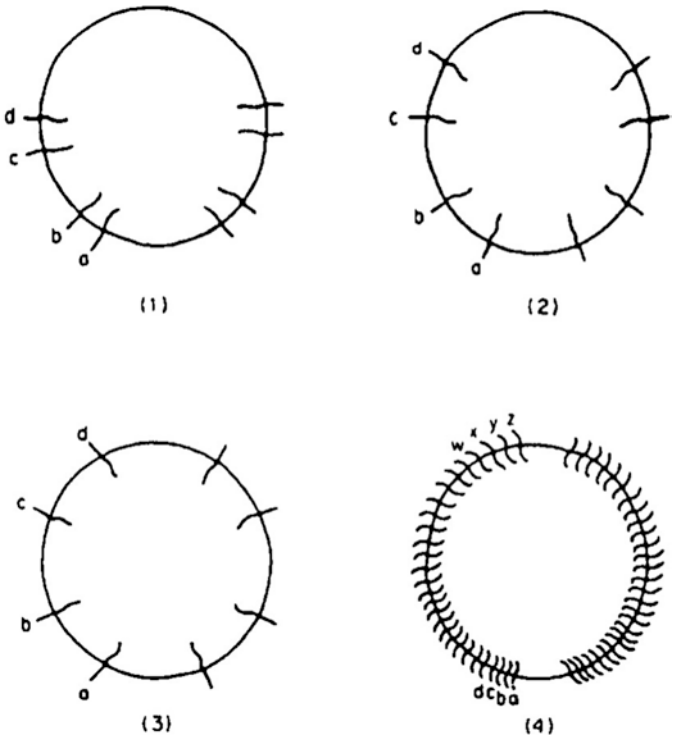

Fig. 1.2 Arrangement of setae in Oligochaeta. 1,2,3, lumbricine arrangement; 4, perichaetine arrangement. 1, Closely paired; 2, widely paired; 3, distant paired

are termed "distant." The distance between each pair of setae and between neighboring pairs is constant for each species. The setae are designated by letters *a*, *b*, *c*, and *d*, beginning with the most ventral setae on each side (Fig. 1.2). The distance between the setae is important as a systemic character and is usually expressed as the ratio of distances between setae. For example, "*aa:ab:bc:cd:dd* = 16:4:14:3:64" is the ratio in the post-clitellar region of *L terrestris*. Alternatively, the distances may be expressed as an equation, *aa* = 4*ab*, or as *ab*<*bc*>*cd*. The distance between the two most dorsal setae on either side is usually compared with the circumference of the body *(*μ*)* at that point, thus *dd* = 1/2μ. For many species of Megascolecidae, particularly *Megascolex*, *Pheretima*, and *Perionyx*, and certain other species, the setal arrangement is termed perichaetine. Here the setae are arranged in a ring right around the segment, although usually with a large or smaller break in the middorsal and midventral regions. (Both types of setal arrangement are shown in Fig. 1.2.) This group of earthworms has more than 8 setae per segment, often 50–100, sometimes more. Setal distributions intermediate between the lumbricine and perichaetine arrangements may be 12, 16, 20, or 24 setae per segment, arranged fairly distinctly in 6, 8, 10, or 12 pairs, or the arrangement may be lumbricine in the

anterior and appears perichaetine in the posterior part of the body. The setae are designated usually by the letters *a*, *b*, *c*, and *d* beginning with the most ventral one on each side, and *z*, *y*, *x*, and *w* beginning with the most dorsal one on each side, irrespective of how many there are in between.

1.3 Genital and Other Apertures

Earthworms are hermaphrodite and possess both male and female genital openings to the exterior, consisting of paired pores on the ventral or ventrolateral side of the body. In lumbricid earthworms, the male pores are situated ventrolaterally on the 15th, or occasionally on the 13th segment. Each pore lies in a slit-like depression, which in some species is bordered by raised lips or glandular papillae, often extending on to the neighboring segments. In other families, the male pores may be on quite different segments, and in some, particularly the Megascolecidae, the male pores may be associated with one or two pairs of prostatic pores; these are openings of the ducts of accessory reproductive bodies known as prostates, which are usually absent from lumbricid species. Both the male and prostatic pores may be on raised papillae or ridges or may open directly onto the surface. The male and prostatic pores are sometimes combined as one opening, but when separate, they are usually joined by longitudinal seminal grooves, on either side of the ventral surface of the body.

Usually, earthworms have two or more pairs of spermathecal pores, with a maximum of seven pairs in some species, but in other species, these are absent (*Bimastos tenuis*, *B. eiseni*). Earthworms belonging to two or three families have only a single pair of pores. Spermathecae and their pores are not always paired. For instance, some megascolecid worms, particularly the genus *Pheretima*, have a single series of pores, situated in the midventral line. The same genus has species with many spermathecae per segment. For example, *Pheretima stelleri* has as many as 30. Spermathecal pores are usually intersegmental and are most often situated in the ventral or latero-ventral position, but sometimes they are close to the middorsal line.

The female pores are most commonly a single pair, situated either in an intersegmental groove or on a segment, their position often being diagnostic of a particular family. Thus, in the related family (Enchytraeidae), they are in groove 12/13, and in the Lumbricidae, Megascolecidae, and Glossoscolecidae, they are on segment 14. Sometimes the female pores are united into a single median pore.

The dorsal pores, which are small openings situated in the intersegmental grooves on the middorsal line, occur in most terrestrial oligochaetes but are not present in aquatic and semiaquatic species. These pores communicate with the body cavity and the coelomic fluid. There are usually no dorsal pores in the first few intersegmental grooves. The position of the first dorsal pore is used as a systematic character at the species level, although these pores may be hard to distinguish in some earthworms. Some species have pores, which are readily visible, and some, e.g., *Aporrectodea rosea*, have pores surrounded by a dark pigmented zone.

The other openings in the body wall are the nephridiopores, which are very small and often difficult to see. They are situated just posterior to the intersegmental grooves on the lateral aspect of the body and usually extend in a single series along the body on either side. The nephridiopores are the external openings of the nephridia (the excretory organs of the earthworm) and, like the dorsal pores, are normally kept closed by sphincter muscles.

1.4 Clitellum and Associated Structures

The clitellum which occurs only in adult earthworms is the glandular portion of the epidermis that is associated with cocoon production. It is either a saddle-shaped or annular structure, the former being most usual among lumbricids. It usually appears swollen, although sometimes it can be differentiated externally only by its color, being paler or darker than the rest of the body, or even a different color. In some megascolecids, it appears only as a well-defined constriction. When swollen, the intersegmental grooves are often indistinct or entirely obscured, particularly on the dorsal surface.

The position of the clitellum and the number of segments over which it extends differ considerably among oligochaetes. Lumbricidae have the clitellum on the anterior part of the body, behind the genital pores, beginning between segments 22 and 38, and extending over about 4–10 segments posteriorly (Fig. 1.3). Megascolecidae have the clitellum further forward, beginning at or in front of segment 14, thus including the female pore and posteriorly it may also include the male pore. Some members of the Enchytraeidae have a clitellum which is only a very temporary development during the period of cocoon formation while even in the Lumbricidae it is often only conspicuous during the breeding phases.

Most earthworms possess various markings at sexual maturity in the form of tubercles, ridges, and papillae on the anterior ventral surface, and these differ greatly in number and form in different species of oligochaetes. The tubercula pubertatis are glandular thickenings on the ventral surface, either on or near the clitellum. Lumbricidae have these in the form of a pair of more or less oval longitudinal ridges, which are sometimes partly divided by intersegmental grooves, or they are separate papillae on either side of the ventral surface of the clitellum. The tubercula pubertatis usually extend over fewer segments than those occupied by the clitellum, except in a few species of lumbricids where they extend beyond the clitellum. Those species without spermathecae, e.g., *Bimastos eiseni*, usually have no tubercula pubertatis. It has been suggested that these organs assist in keeping earthworms together during copulation. Earthworms of other families do not always have the tubercula pubertatis situated on the clitellar segments, for example, in the Megascolecidae; they are situated on either side or on both sides of the clitellum, or on the clitellar segments. They may be transverse ridges or small separate pads, usually symmetrically (or sometimes asymmetrically) arranged about the midventral line.

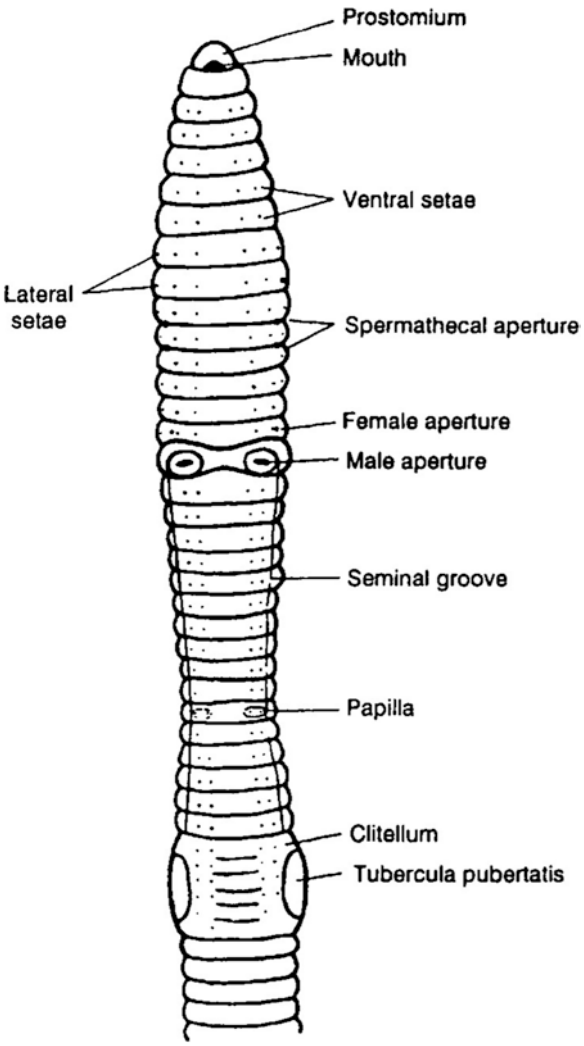

Fig. 1.3 Ventral view of the anterior region of *L. terrestris*

The position of the clitellum is used as a diagnostic character, particularly for lumbricids, because the position and number of segments occupied by the clitellum are, with small variations, constant for each species. Its position is defined by the number of the first and last segments occupied, with the range of variations in position, as, for example, 26, 27–31, and 32. Clitellum has been used as indicators of soil contaminants such as but not limited to hydrophobic organic contaminants (Shi et al., 2018).

1.5 Pigmentation and Coloration

Earthworms have a wide range of colors, and many are iridescent. The color of earthworms depends mostly on the presence or absence of pigment, which is either in the form of granules or in pigmented cells in the subcuticular muscle layer. Pigmented earthworms, such as L. *terrestris*, are usually red, brown, a combination of these colors, or even grayish or greenish. Occasionally, the color is at least partly due to the presence of yellow coelomic fluid or green chloragogenous cells near the body surface. The ventral surface of these earthworms is usually much lighter in color than the dorsal surface, although some deeply pigmented megascolecids are pigmented equally on both dorsal and ventral surfaces. The pigments, which are mainly porphyrins, probably originate as breakdown products of the chloragogenous cells (Stephenson, 1930). Unusually, the pigment is not evenly distributed but appears as dark segmental bands separated by lighter intersegmental zones, as in some strains of *Eisenia fetida*. Lightly pigmented or unpigmented worms often appear reddish or pink, due to the hemoglobin of the blood in the surface capillary vessels showing through the transparent body wall. If the body wall is opaque, unpigmented worms are whitish. In a number of species, the cuticle is strongly iridescent and causes the earthworms to appear bluish or greenish. This is particularly noticeable in species of *Lumbricus* and *Dendrobaena*. The color of pigmented worms, when preserved in formalin, is fairly stable, but the reds and pinks of unpigmented earthworms usually fade rapidly.

1.6 Body Wall

The body wall consists of an outer cuticle, the epidermis, a layer of nervous tissue, circular and longitudinal muscle layers, and finally the peritoneum, which separates the body wall from the coelom. The cuticle, which is a very thin (7 μm in *L. terrestris*), noncellular layer, is colorless and transparent, consisting of two or more layers, each composed of interlacing collagenous fibers with several homogeneous nonfibrous layers beneath. It is perforated by many small pores which are larger and more irregularly distributed about the middle of each segment than in its anterior part and are absent from the posterior part of each segment. The cuticle is thinnest where it overlays the epithelial sense organs, and here it is perforated by many very small pores through which project fine hairs from the sensory cells.

The epidermis consists of a single layer of several different kinds of cells (Fig. 1.4). The supporting cells, which are columnar in shape, are the main structural cells of the epidermis and have processes which extend into the muscle at their bases. These cells secrete material to form the cuticle. Short and round or club-shaped basal cells lie on the inner wall of the epidermis and are also known as "replacing cells," although it is doubtful whether they can replace the epithelial cells. These characteristics offer potentials for the epidermis in bioremediation of

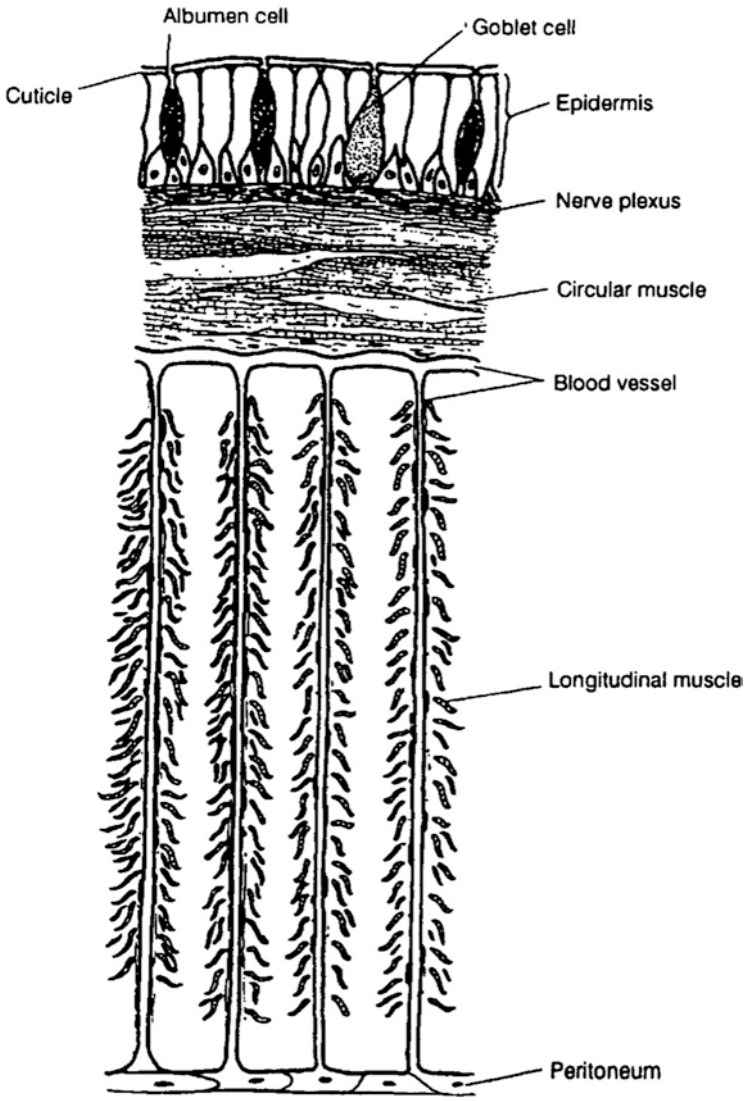

Fig. 1.4 Transverse section of a portion of the body wall

recalcitrant contaminants. For instance, Zhang et al. (2021a) showed that earthworm epidermis transformed by nZVI (nano-zero valent iron, an iron used for groundwater remediation) and undergoing oxidative stress and lipid peroxidation could actually enhance the bioaccumulation of PCB77 through the epidermis layer.

Two forms of glandular cells present are the mucous or goblet cells and albumen cells. Mucous cells secrete mucus over the surface of the cuticle to prevent desiccation and to facilitate movement of earthworms through soil. The function of the albumen cells is not known.

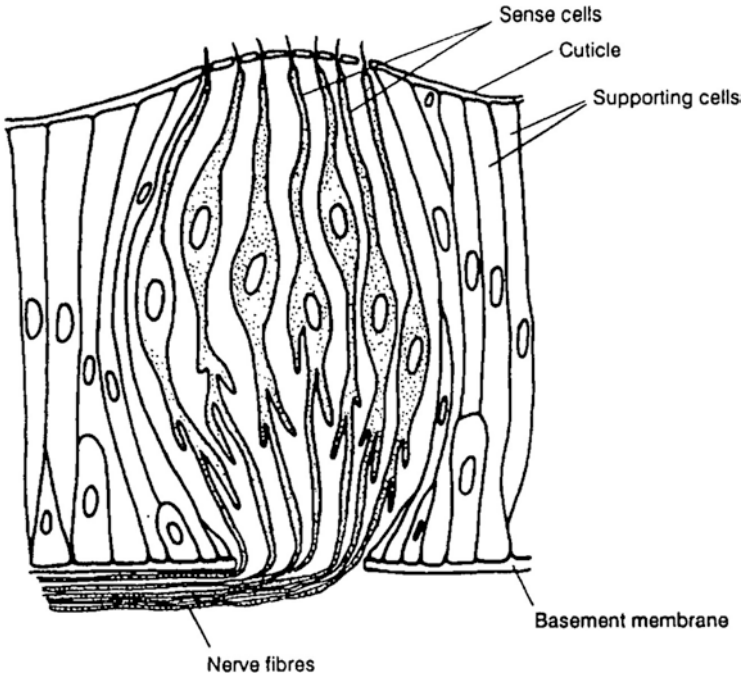

Fig. 1.5 Vertical section of an epidermal sense organ. (After Grove & Newell, 1962)

Large numbers of sensory cells, grouped together to form sense organs which respond to tactile stimuli, are scattered throughout the epidermis (Fig. 1.5). These are more numerous on the ventral than on the dorsal surface. Photoreceptor cells, capable of distinguishing differences in light intensity, occur in the basal part of the epidermis and are most numerous on the prostomium and first segment and on the last segment. There are few or none in the epidermis of the middle segments. The epithelium of the buccal cavity bears groups of sensory cells which can be stimulated by chemical substances associated with taste. The prostomium contains receptors which can detect sucrose, glucose, and quinine, as well as many other chemicals.

The epidermis is bounded at its inner surface by a basal membrane within which lie two muscle layers. The circular muscle layer consists of muscle fibers extending around the circumference of the body, except at the intersegmental positions. The muscle fibers are arranged in an irregular manner, aggregated into groups, each surrounded by a sheath of connective tissue (Fig. 1.4).

The longitudinal muscles, which are in a much thicker layer than the circular muscles, are continuous throughout the length of the body and arranged in groups or blocks around the body. *Lumbricus* has nine such blocks of muscles: two dorsal, one ventral, and three ventrolateral on either side. They have ribbon-like fibers which are arranged into U-shaped bundles in each block; these are surrounded by sheaves of connective tissue, so that the mouth of the "U" is toward the coelom.

Usually the ends of the "U" are closed, forming a box-like structure, but some species, e.g., *Eisenia fetida*, have both ends open, so that the bundles are disposed in radial columns. Between each bundle lies a double layer of connective tissue containing blood vessels. The inner surface of the longitudinal muscle layer is separated from the coelom by a layer of coelomic epithelial cells, the peritoneum.

1.7 Coelom (Body Cavity)

The coelom is a large cavity that extends through the length of the body and is filled with coelomic fluid. It is surrounded on the outer side by the peritoneum of the body wall and on the inner side by the peritoneum covering the alimentary canal. Transverse septa divide it into segmental portions. The peritoneum covering these septa is similar in structure to that covering the inner surface of the muscle layers; in a few species, the peritoneum on the septa is so very much thickened that it almost fills the coelom in this region. The septa usually correspond to the external segmental grooves but often do not occur in the first few segments of the body, and in some species, they are missing in other parts such as the esophageal region of some species of *Pheretima*. Some species have septa that do not correspond with the intersegmental grooves in the anterior end but are displaced backward, sometimes by as much as a segment. Two adjacent septa are sometimes fused at their junction with the body wall. The septa differ in thickness, depending on their position in the body, those in the anterior of the body being markedly thickened and more muscular. The degree of thickening and the position and number of these septa are used as systemic features for identifying many species of earthworm. For example, a species of earthworm recently discovered near the Muling River of Heilongjiang Province, Northeast China (adjacent to Russia), possesses particularly thick and muscular septa with its slightly yellowish, small, heart-shaped ampulla attached to septum $^{7}/_{8}$ (Zhang et al., 2021b). This species of earthworms also has a characteristic single pair of testis sacs which elongate to the middle part of septum 9/10. Septa are constructed from muscle fibers, usually derived from the longitudinal muscle layer, together with some circular muscles on the posterior face with connecting tissue and blood vessels. The septa are usually perforated by pores which allow the coelomic fluid to pass freely between segments, although some septa effectively isolate each body segment and make it virtually self-sufficient with its own supply of blood, central and peripheral nervous system, excretory system, and even gonads.

There are also bands and sheets of mesenteric membranes between the body wall and the gut, forming pouches and dividing off some organs into separate chambers. Some peritoneal cells are modified in the region of the intestine as chloragogen cells, forming chloragogenous tissue. Chloragogenous cells are characterized by the presence of yellow or greenish-yellow globules, the chloragosomes.

The coelomic fluid is a milky white liquid which is sometimes colored yellow by eleocytes (cells containing oil droplets) as in *Dendrobaena subrubicunda*. The

coelomic fluid of *E. fetida* smells similar to garlic, hence the name of this species. The consistency of the coelomic fluid differs between different species of earthworms and also depends upon the humidity of the air in which the earthworms live. Thus, it is thicker and more gelatinous in earthworms in dry situations than in those from wetter habitats.

The coelomic fluid contains many different kinds of particles in suspension. The inorganic inclusions are mainly crystals of calcium carbonate, but the corpuscular bodies in the coelomic fluid of lumbricid earthworms include phagocytic amebocytes, which feed on waste materials, vacuolar lymphocytes (small disk-shaped bodies which do not occur in earthworms with large numbers of eleocytes), and mucocytes (lenticular bodies which give the coelomic fluid a mucilaginous component). Other inclusions in the coelomic fluid include breakdown products of the corpuscular bodies, protozoan and nematode parasites, and bacteria. "Brown bodies" are aggregated dark-colored masses or nodules usually found in the coelom at the posterior end of the body. They consist of disintegrated solid debris such as setae and the remains of amebocytes and also cysts of nematodes and *Monocystis*.

Many earthworms eject coelomic fluid through the dorsal pores in response to mechanical or chemical irritation or when subjected to extremes of heat or cold. Some species, such as *Megascolides australis*, can eject fluid to a height of 10 cm, and *Didymogaster sylvaticus* (known as the squirter earthworm) to a height of 30 cm. Coelomic fluid is also expelled through the dorsal pores at times of stress and may have several functions such as preventing desiccation, promoting cutaneous respiration, or providing protection from predators (Vail, 1972).

As with many other earthworm organs, the status of the coelom tissues may be used to assess contamination. Tang et al. (2020) reported that low-level arsenic contamination can damage coelom tissues resulting in overaccumulation of high As concentrations in the earthworm hindgut. This, along with the significant increase in betaine levels and a decrease in dimethylglycine and myo-inositol levels in earthworms, suggests that the osmoregulatory metabolism of earthworms may have been disturbed.

1.8 Alimentary Canal

The alimentary canal or gut of earthworms is basically a tube extending from the mouth to the anus although it is differentiated into a buccal cavity, pharynx, esophagus, crop, gizzard, and intestine (Fig. 1.7). The short buccal cavity flanked by a tube-like prostomium begins at the mouth and occupies only the first one or two segments with one or two diverticula or evaginations. The epithelium lining the buccal cavity is not ciliated, except occasionally in a small dorsal diverticulum, though very young earthworms can have patches of ciliated epithelium on the ventral surface. Overlying the epithelium, except in the ciliated portions, is a thick cuticle.

The pharynx, which is not always differentiated clearly from the buccal cavity, extends backward to about the sixth body segment. The dorsal surface of the

pharynx is thick, muscular, and glandular and contains the pharyngeal glands, which secrete mucus and appear as a whitish lobed mass. Some species, e.g., *Bimastos parvus*, have pharyngeal glands which extend back beyond the pharynx and are attached to the septa. Earthworms use the pharynx as a suction pump, so that muscular contractions of its walls draw particles through from the mouth, and some species evert the pharynx in this process.

All terrestrial oligochaetes have an esophagus, which opens from the pharynx as a narrow tube, modified posteriorly as a crop and gizzard. Calciferous glands situated in evaginations or folds in the esophageal wall open into the esophagus. Lumbricidae have these folds developed strongly with their tips fused together, to form esophageal pouches, which separate the glands from the esophageal cavity. Esophageal pouches are usually paired and lateral but may be unpaired and ventral in some megascolecid species. In *Pheretima* spp., they are reduced to low ridges of calciferous tissues. In some species of lumbricids, such as *Lumbricus castaneus*, *Lumbricus rubellus*, and *Dendrobaena* spp., the glands connect with the lumen of the esophagus via a duct.

At the posterior end of the esophagus is the crop, a thin-walled storage chamber situated in front of the gizzard, which is muscular and lined with a thicker cuticle than the crop. Muscular contractions of the gizzard grind up the food into small particles with the aid of mineral particles taken in with the food. The crop and gizzard are situated much further forward in some of the other species of earthworms than in the Lumbricidae; they are immediately behind the pharynx in megascolecid species which commonly have two to ten gizzards, each occupying one segment.

One species of megascolecid, *Pheretima californica*, has no gizzard and a crop situated in the middle of the esophagus. It has no calciferous glands in the strict sense of the term, although there are folds in the wall of the posterior half of the esophagus. The intestine has two conical caecae which originate in segment 26 and extends into three or four segments (El-Duweini, 1965). Megascolecids are extremely variable in terms of gizzard development, some species having as many as ten gizzards.

The rest of the alimentary canal is the intestine, which is a straight tube for most of its length, constricted slightly at each septum. Most of the digestion and absorption of food materials take place in the intestine. The internal surface of the intestine has many small longitudinal folds, and its surface area is increased by a large fold in the medial dorsal line, the typhlosole, which projects from the dorsal wall (Fig. 1.6). This differs in relative size in different species, being largest in the lumbricid earthworms. Such increases in intestinal surface area are common in animals that consume food mostly of plant origin, but the typhlosole may be small in some other species.

The epithelial lining of the intestine is composed mainly of glandular cells and non-glandular ciliated cells. The ciliated cells of *Lumbricus* species are also contractile, acting upon the gland cells which they surround, causing them to open or close. The intestine has two muscular layers, an inner circular and an outer longitudinal layer, the reverse order to that in the body wall.

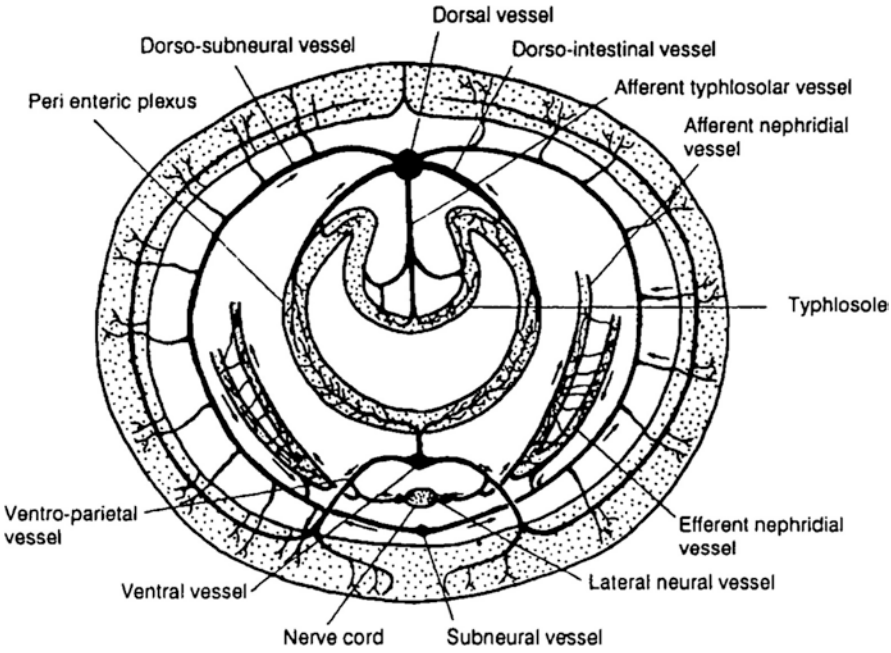

Fig. 1.6 Diagram to show the main segmental blood vessels in the intestinal region

1.9 Vascular System

Earthworms have a closed vascular system, although it is not possible to distinguish "arteries" and "veins." Instead, there is a system of vessels that distribute blood in various directions. There are three principal blood vessels, one dorsal and two ventral, that extend almost the entire length of the body, joined laterally in each segment by blood vessels which ring the peripheral region of the coelom and the body wall (Figs. 1.6, 1.7, and 1.8). The largest of the longitudinal vessels, the contractile dorsal vessel, is associated closely with the gut for most of its length, except in the most anterior portion, where it is separated from the gut by a mesentery. Some species of Megascolecidae and Glossoscolecidae have paired dorsal vessels for part or all of the length of the body. The ventral vessel, which is narrower than the dorsal vessel, lies immediately below the gut and is suspended from it by a mesentery. It conveys blood from the anterior part of the body to the posterior. The subneural vessel is even smaller than the ventral vessel. It lies beneath the ventral nerve cord, with which it is closely associated for its entire length. Some earthworm species do not have a subneural vessel, but most species (except some Megascolecidae) have two much narrower vessels that lie either side of the ventral nerve cord throughout its length (the latero-neurals). Megascolecid and some glossoscolecid earthworms, but not lumbricids, have a supra-intestinal vessel (some genera, e.g., *Pheretima*, have

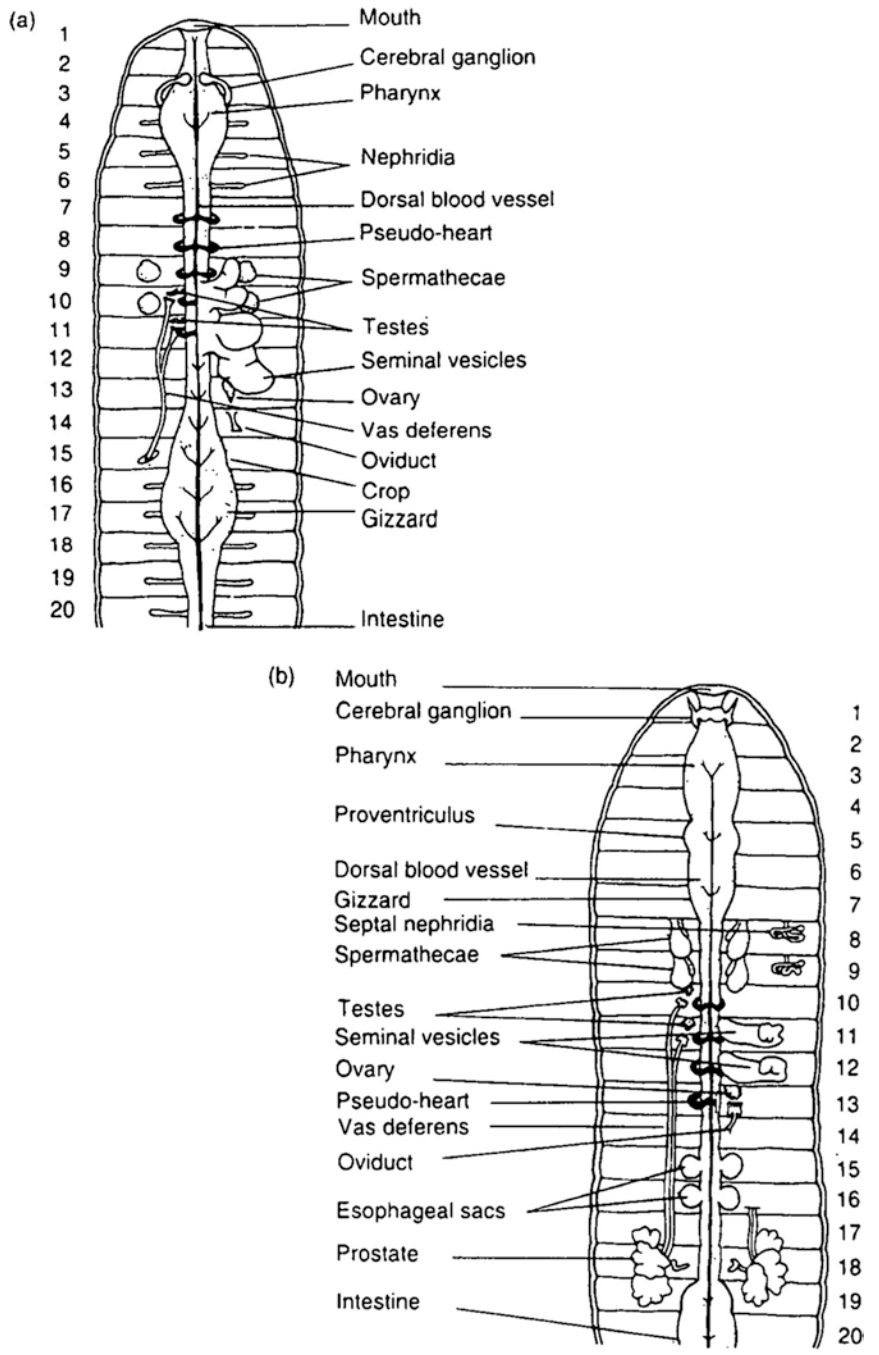

Fig. 1.7 Digestive, circulatory, and reproductive systems of earthworms (♂/♀reproductive structures not bilaterally complete; all nephridia not shown). (**a**) Diagrammatic lumbricid (based on *L. terrestris).* (**b**) Diagrammatic megascolecid (based on *Pheretima* spp.)

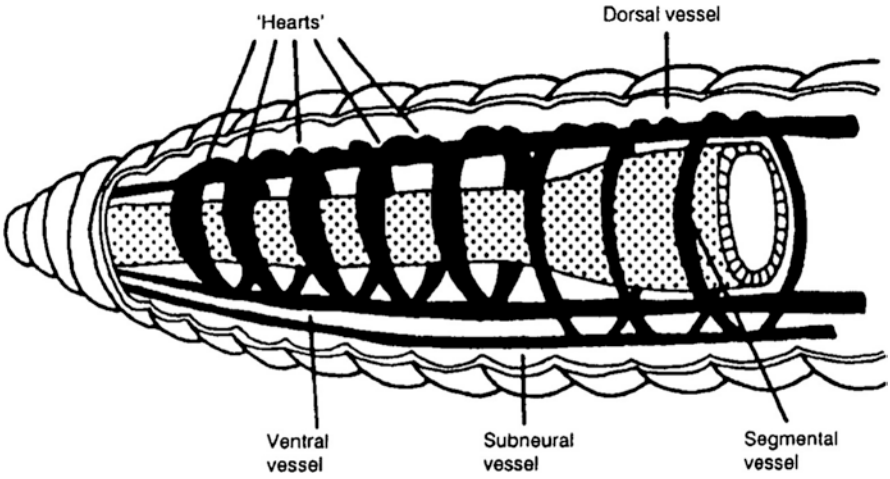

Fig. 1.8 (**a**, **b**) The circulatory system of a lumbricid

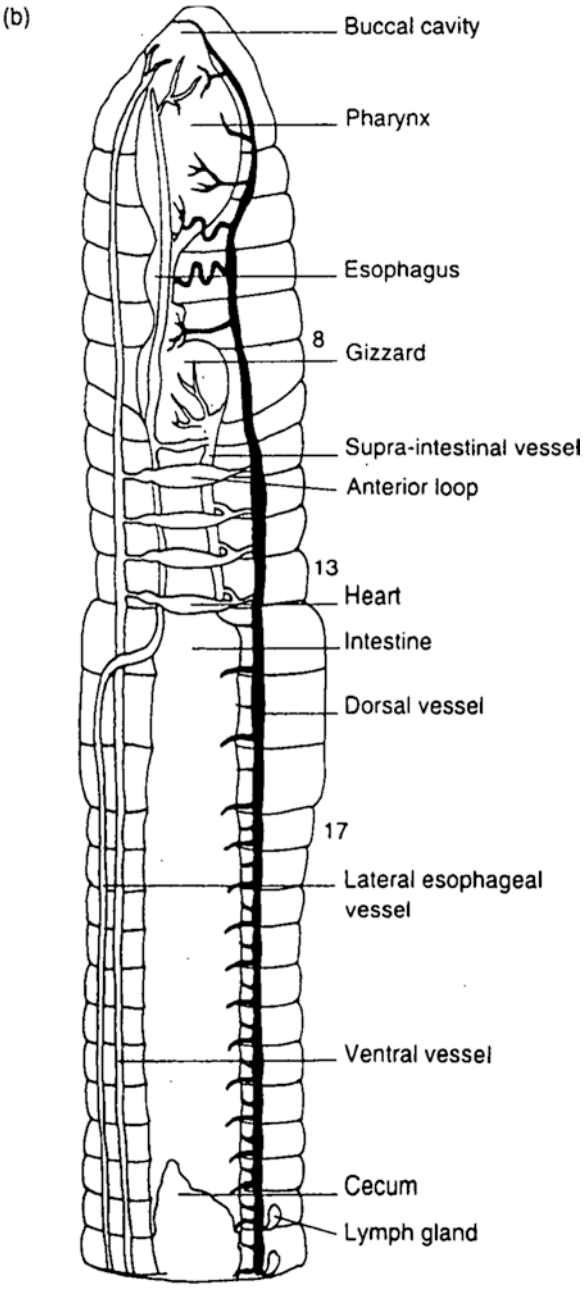

Fig. 1.8 (continued)

two such vessels); this lies along the dorsal wall of the gut in the anterior segments and is part of the complex of blood vessels serving the alimentary canal. Other longitudinal blood vessels are the extra-esophageal (or lateral-esophageal) vessels which lie along either side of the gut, from the pharynx to the first of the dorso-subneural vessels.

The paired commissural blood vessels pass round the body in each segment from the dorsal vessel (and supra-intestinal vessel in those species which have it) to the ventral or subneural vessels. Some of the anterior commissures are enlarged, contractile, and with valves termed "hearts" or "pseudo-hearts"; *Lumbricus* has five pairs of such vessels (situated in segments 7–11), but some species have more and others less; megascolecid worms have between two and five pairs of "hearts." For instance, *Pheretima californica* has four "hearts." In addition to these dorso-ventral anterior "hearts," some species have "intestinal hearts" which connect the supra-intestinal vessels with the ventral vessel, and also other "hearts" originating in branches from the dorsal and supra-intestinal vessels; *Pheretima* has "hearts" of both these types. The "hearts" direct the flow of blood through the body by means of valves similar to those in the dorsal vessel. The lateral-esophageal-subneural commissures link the lateral-esophageal and subneural vessels in the anterior segments of the body. Behind segment 12, a pair of dorso-subneural commissures, lying on the posterior face of each septum, link the dorsal vessel with the subneural vessel in each segment. A pair of ventro-parietal vessels on the anterior face of each segment branch off from the ventral vessel to the body wall and end in capillaries. Some species of Megascolecidae have ventral-supraintestino-commissures. The commissural or septal vessels are always associated with the faces of septa, and sometimes the "hearts" are attached by mesenteric-like folds of the peritoneum to the faces of the septa. Between the alimentary epithelium and the outer muscular and peritoneal layers is a vascular network or plexus, the perienteric or alimentary plexus, which communicates with the dorsal and ventral vessels by two pairs of dorso-intestinal and three pairs of ventro-intestinal vessels per segment. The dorsal vessel supplies blood to the typhlosole via three small typhlosolar vessels per segment.

The body wall has capillaries which follow the muscle bundles and eventually connect with the subneural vessel via the lateral-esophageal-subneural commissures in the front of the body, or via the dorso-subneural commissures in the rear. These capillaries also connect with the supra-intestinal vessel and the supra-intestino-ventral commissure behind the 12th segment of *Lumbricus*. The paired dorso-subneural commissures run between the dorsal and subneural vessels in every segment behind the 12th.

The efferent nephridial vessels join the dorsal-subneural, and the smaller and more numerous afferent nephridial vessels come from the ventro-parietals. The reproductive organs receive most of their blood supply from the ventro-parietal vessels.

1.10 Respiratory System

Terrestrial oligochaetes have few specialized respiratory organs. Most respiration is through the body surface which is kept moist by the mucous glands of the epidermis, the dorsal pores of which exude coelomic fluid and the nephridial excretions through the nephridiopores.

This method of gaseous exchange depends upon a network of small blood vessels buried in the body wall of terrestrial earthworms, so that oxygen dissolved in the surface moisture film can permeate through the cuticle and the epidermis to the thin walls of these vessels, where it is taken up by the hemoglobin in the blood and passed around the body. The hemoglobin in earthworms has a very high affinity for oxygen. As in other animals, carbon monoxide can block the functioning of hemoglobin in earthworms (Gardiner, 1972). Some species of *Lumbricus* also have looped capillaries extending from the ventro-parietal blood vessels into the epidermal layer. *Aporrectodea caliginosa* f. *trapezoides* has special respiratory regions of the body wall, where the cuticle is thinner, the epithelial cells shorter, there are fewer glandular cells in the epidermis, and there is a special subcutaneous blood supply. In order for the body surface to act as a respiratory organ, it has to be kept moist; this is achieved by secretions mainly from mucous glands. The mechanism for removal of carbon dioxide from the earthworm body is poorly understood. Carbonic acid formed in the calciferous glands can combine with calcium and pass out of the body with the feces. The high affinity of earthworm hemoglobin for oxygen allows earthworms to live even in poorly ventilated soils. However, when soil is flooded after rain, earthworms may experience low oxygen tensions. This may cause them to leave their burrows in search of atmospheric oxygen. The rate at which respiration occurs may also be affected by factors such as soil contamination. Wang et al. (2021) confirmed that an outsize contamination of arsenic in formerly industrialized land in China could inhibit the rate of earthworm respiration.

1.11 Excretory System

The following description of the excretory system refers to *L. terrestris*, and detailed differences in other species are discussed later. The nephridia, which are the main organs of nitrogenous excretion in oligochaetes, are paired in each segment except the first three and the last. The internal opening from the coelom into each nephridium is just in front of a septum and is a funnel-shaped nephrostome (Fig. 1.9a, b) which leads to a short pre-septal canal that penetrates the septal wall into the segment behind, where the main part of the nephridium lies. The nephridium continues as a long post-septal canal with three loops, which can be distinguished as four sections: a very long "narrow tube," a shorter ciliated "middle tube," a "wide tube," and, finally, the "muscular tube" or reservoir, which opens to the exterior at the nephridiopore (Fig. 1.10). In addition to functioning as an important component of

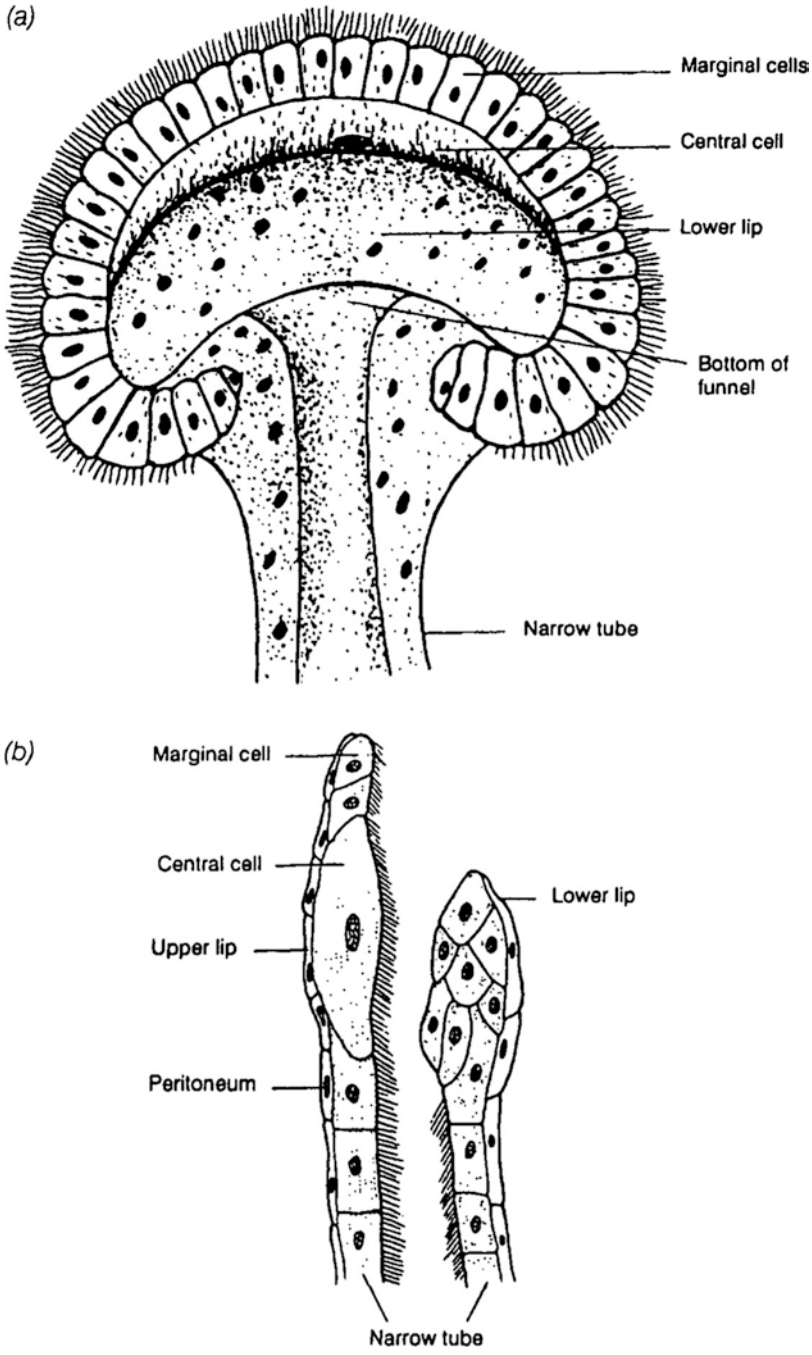

Fig. 1.9 Nephrostome: (**a**) ventral view; (**b**) longitudinal section

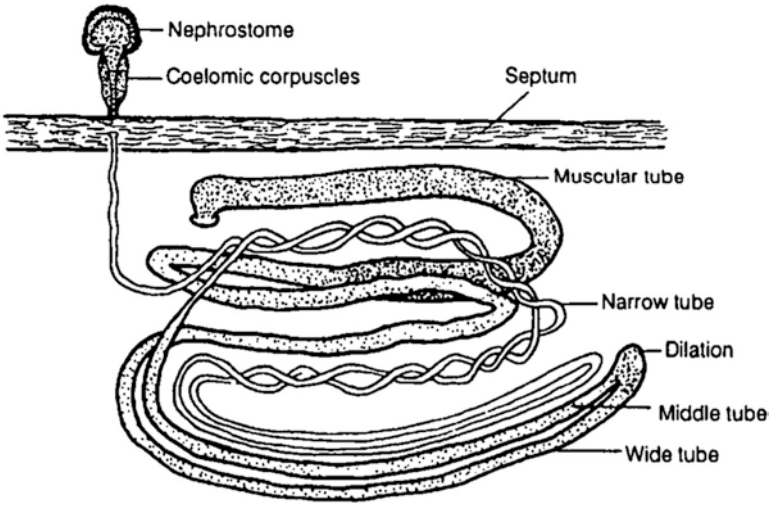

Fig. 1.10 Diagram of a nephridium

the earthworm excrement system, the nephridial cells also play a role in eliminating invading microorganisms by means of excretion which culminates in further expulsion through the dorsal pores (da Cruz Hung et al. 2021).

The mouth of the nephrostomal funnel is flattened into two lips, an upper posterior lip and a lower anterior lip. The upper lip is much larger than the lower lip and forms a semicircular expansion overhanging and projecting beyond the lower lip (Fig. 1.9b). The upper lip is composed of a radiating circle of marginal cells that are ciliated on their edges and inner faces, the cilia beating toward the canal. The lip is peripheral to a large crescent-shaped central cell with nucleate marginal cells and cilia on its lower face. The dorsal wall of the pre-septal canal extends to the lower boundary of the central cell, forming a part of the upper lip which has no nuclei or cilia; neither has the lower lip, which is much thinner than the upper lip. The short pre-septal canal, which leads from the nephrostome to the wall of the associated septum, is intercellular, like most of the nephridial tubes, i.e., the lumen perforates a single column of cells which are called "drainpipe cells," and which have cilia in two longitudinal rows, one on each lateral wall.

Most of the post-septal part of the nephridial tube is secretory, except for the last portion (the muscular tube), and the lumen of the whole of the secretory section is intracellular, also with "drainpipe cells." The first part of the post-septal canal is the "narrow tube," which has thin transparent walls containing granular protoplasm, nucleated at intervals (Fig. 1.10). The only parts of this tube that are ciliated are at the beginning, about halfway along its length and at the end. After the narrow tube is the "middle tube," which is shorter and wider, with thicker brown-colored walls, and two rows of cilia along its whole length. This leads into the unciliated "wide tube," which begins at a point where one of the loops bends sharply back on itself, via the ampulla, a dilated chamber with very large "drainpipe" cells. The inner

surface of the ampulla is covered with a large number of rod-like bacteria, and the last part of the wide tube has a ringed appearance. The last part of the nephridial duct is the "muscular tube," which is much wider than the preceding tubes, is unciliated, and is the only part to have an intercellular lumen. This tube has a lining of flat epithelial cells surrounded by a muscular coat in its wall. It opens to the exterior by the nephridiopore through a sphincter muscle.

Nephridia of this pattern, i.e., opening externally on to the body surface (exonephridia) are found in most lumbricids, but in *Allolobophora antipae*, all the nephridia from segment 35 backward open into two longitudinal canals that discharge into the posterior part of the gut (enteronephridia) (Fig. 1.11a). The system in *Megascolex* spp. is similar. The nephridia of *Lampito mauritii* empty into a median excretory duct which leads to the posterior portion of the intestine (Fig. 1.11b).

Large, paired nephridia such as described above, are known as meganephridia, but some earthworms also have micronephridia, which are much smaller and more numerous. For instance, species of *Pheretima* have 40–50 micronephridia attached to each septum, except those septa in the anterior part of the body. Micronephridia are much simpler in form and hang freely in the coelom from the ends of their

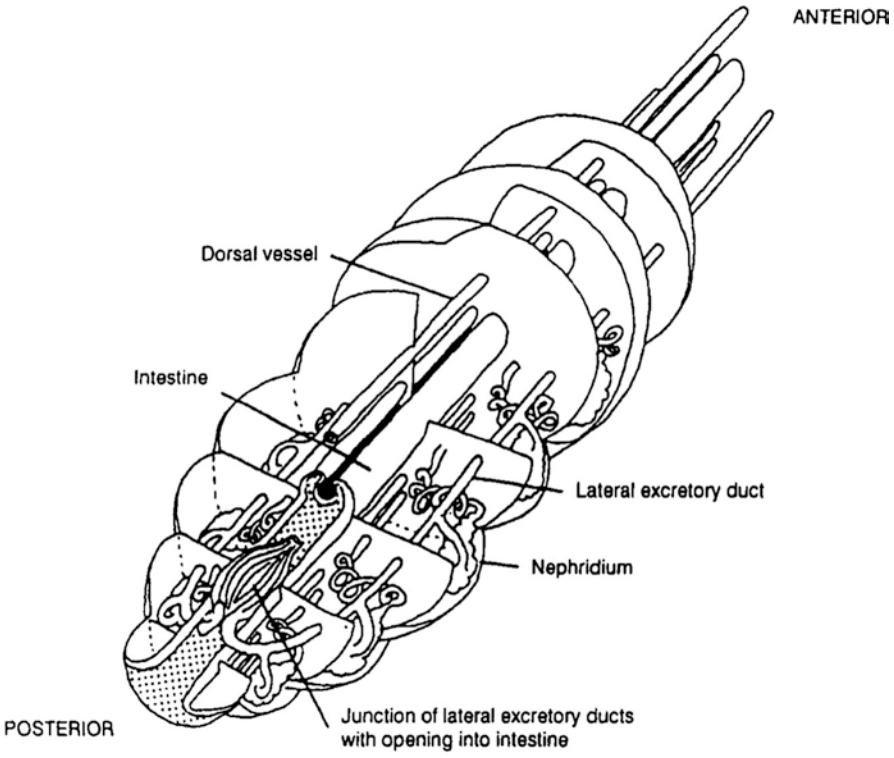

Fig. 1.11 (**a**) The excretory system of *Allolobophora antipae* (**b**) The excretory system of *Lampito mauritii*

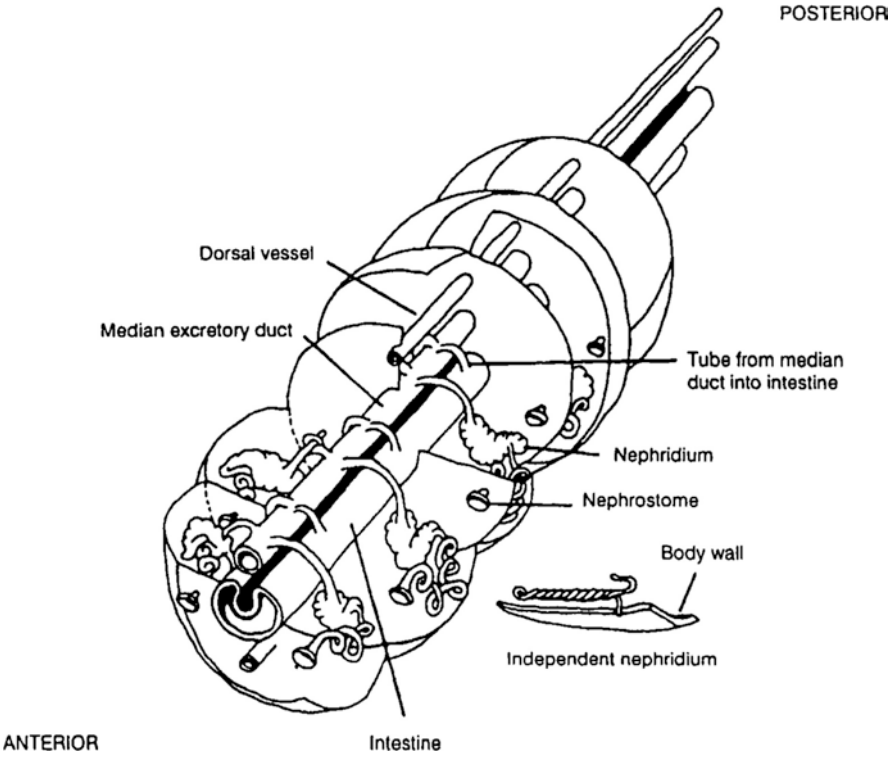

Fig. 1.11 (continued)

funnels. Leading from a short, narrow tube is the main part of the micronephridial tube, a long, spirally twisted loop which has a ciliated lumen only in certain parts. The terminal ducts of these tubes pass into two septal excretory canals which curve around the posterior faces of the septa and then pass to supra-intestinal excretory ducts that lie on either side of the dorsal surface of the gut. Some other megascolecid worms also have exonephric micronephridia.

Other kinds of nephridia end in a closed tube instead of a nephrostome; they are very small, exonephric, and attached to the body wall, with as many as 200–250 per segment. Species of *Pheretima* have this type of nephridia as well as septal micronephridia. They also have tufted nephridia, which are bush-like organs consisting of bunches of nephridial loops on the walls of the alimentary canal in segments 4, 5, and 6, one pair per segment, the ducts from the loops joining up and emptying into the alimentary canal. Some megascolecid species have tufted nephridia throughout the body; other species have them only in parts of the body. All earthworms have nephridia of one or more of the types described above, but the majority of species have open-funneled exonephric meganephridia similar to those of *Lumbricus*.

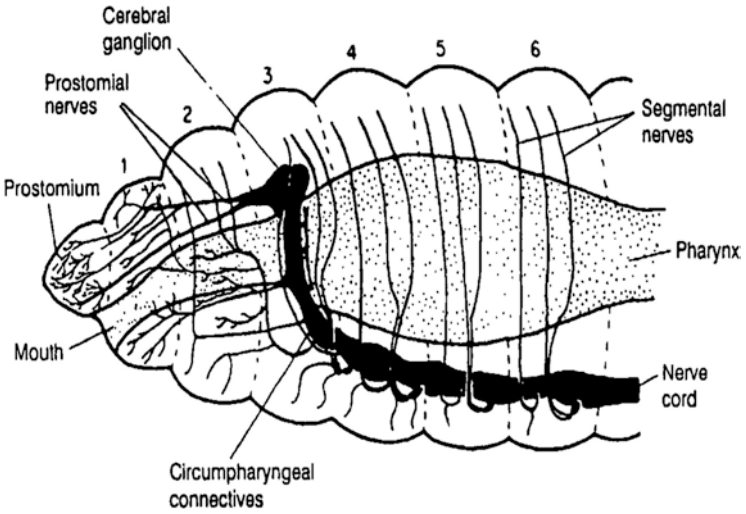

Fig. 1.12 Nervous system of *L. terrestris* from the side

The terms meganephridia and micronephridia are purely descriptive but are disliked by some taxonomists who prefer the terms holonephridia and meronephridia. Holonephridia are always single and relatively few in number in each segment. Meronephridia are multiple, although they are derived from a single rudiment in each segment. They are usually very numerous in each segment and much smaller than holonephridia. The urine of earthworms may contain ammonia, urea, and, to a limited extent, uric acid in different proportions according to species, the season, and the diet of the earthworm.

1.12 Nervous System

Earthworms are very sensitive to chemicals, to touch and to light, and react to avoid adverse conditions. The ventral nerve cord runs beneath the gut close to the ventral wall of the coelom, from the last segment of the body to the fourth segment from the front. Anteriorly, it passes into a subesophageal ganglion and then bifurcates into the circumpharyngeal (circumesophageal) connectives, which pass up around either side of the esophagus and meet as the bilobed cerebral ganglion on the dorsal surface of the pharynx in segment 3 (Fig. 1.12). Structurally, the ventral nerve cord is really a backward extension of the two circumpharyngeal connectives fused together. The ventral nerve cord is swollen into segmental ganglia in each segment and, from the fifth segment backward, each of these ganglia has two pairs of segmental nerves branching from it in the posterior part of each segment, with a third pair just in front of each ganglion. These segmental nerves extend around the body

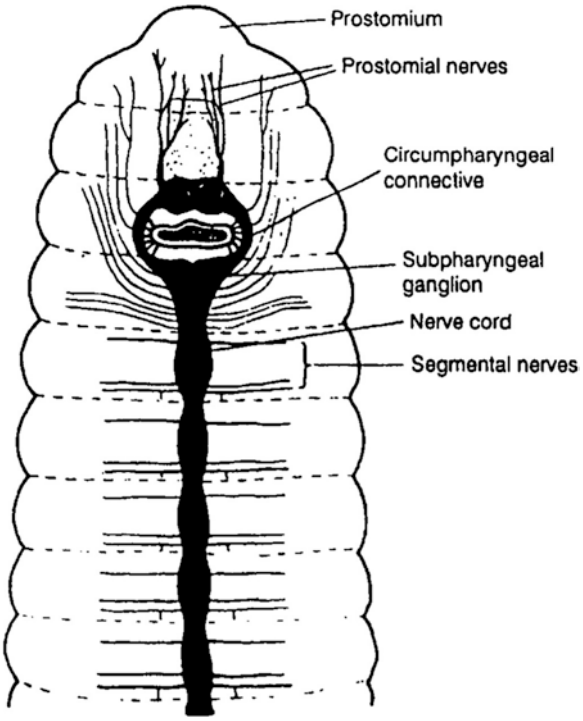

Fig. 1.13 Nervous system of *L. terrestris* from above

wall, at first in the longitudinal muscle layer, and then in the circular muscle layer, ending near the middorsal line, thus forming almost a complete ring (in some megascolecid species, e.g., *Pheretima*, the ring is completely closed). Each nerve ring has branches to the muscles and to the epidermis. Every segmental nerve has a small subsidiary branch, close to the nerve cord, which passes down into the circular muscle layer, and then between this and the longitudinal muscle layer, ending in branches to the epidermis just before it reaches the midventral line. Each septum is supplied by a pair of nerves (septal nerves) arising near the junction of the posterior segmental nerves and the ventral nerve cord (Fig. 1.13).

The distribution of the nerves in the anterior four segments of the body differs from that in the other segments. Segment 3 has the typical distribution of three pairs of segmental nerves, but they originate from segment 4, which also possesses three pairs, so that six pairs of segmental nerves come from the ventral nerve cord in segment 4. Segment 2 is supplied with two pairs of nerves which originate from the junction of the circumpharyngeal connectives with the ventral nerve cord in segment 3, the larger posterior pair dividing, to give the normal three nerve rings in segment 2. The first segment is supplied by a pair of nerves originating in the lateral portions of the subpharyngeal connectives, which branch into two, shortly after they leave these nerves, and ramify through the first segment without forming nerve

rings. A small nerve comes from the most ventral of these two branches to supply the ventral surface of the buccal cavity. The prostomium is innervated by two prostomial nerves, which originate from the front of the cerebral ganglion; these are the only nerves coming from this ganglion. A branch is given off from each prostomial nerve to supply the roof of the buccal cavity. The prostomial nerves then ramify through the prostomium, ending in the prostomial epithelium as nodule-like enlargements which then become part of the subepidermal nerve plexus. The last (caudal) segment of *Lumbricus* normally has six pairs of septal nerves instead of three (Hess, 1925), arranged as if they were in two successive normal segments. The most posterior of these six pairs of nerves are lateral terminations of the nerve cord itself.

The system of nerves supplying the gut is sometimes called "the sympathetic system" and consists of a plexus of nerves which lie between the epithelium and the circular muscle layer of the alimentary canal throughout its length. Six small nerves from each circumpharyngeal connective unite in the external wall of the pharynx as a pharyngeal ganglion, from which nerve fibers pass forward and backward into the plexus. The muscles of the intestinal wall are supplied from a nerve plexus in the septum in each segment.

The wall of each blood vessel is covered with a network of nerve fibers and cells which supply the major blood vessels, such as the dorsal and ventral vessels.

Before bifurcating, branches of the lateral nerves which link with the septal plexuses supply the longitudinal muscles of the body wall. The circular muscles are supplied by branches from nerve rings embedded in the body wall, which contribute to an intermuscular nerve plexus. Between the basal membrane of the epidermis and the circular muscle layer is the subepidermal nerve plexus, similar to the subepithelial plexus in the wall of the alimentary canal. Nerve fibers from this plexus, which is linked with branches from the nerve ring, end between the epidermal cells, or enter the sensory cells. The peritoneum has an extensive nerve net, derived from the nerves of the body wall.

1.12.1 Fine Structure of the Nervous System

The ventral nerve cord is covered externally by a peritoneum, and immediately beneath this is a layer of longitudinal muscle fibers within which lies a thin layer of tissue described as a neural lamella. Beneath these layers, surrounding the nervous tissue, is the fibrous epineurium, which extends inward into the nervous tissue and also outward into the lateral nerves. This layer, which is ectodermal in origin, is made up of cells that resemble connective tissue. Three dorsally situated giant fibers, one median and two lateral, are separated from each other and from the rest of the nervous tissue by fibrous septa or lamellae. Some *Lumbricus* species have two other smaller giant fibers in the ventral portion of the cord. There is also a double vertical septum dividing the cord into two lateral halves, which communicate in each segment by three large fenestrae through which pass fibrillae. The central portions of the nerve cord are partitioned further by extensions of the main horizontal and

vertical septa. The nervous tissue in the cord is ramified by supporting tissue, called the neuroglia, which has both protoplasmic and fibrous cells, and a close network of fibers derived from processes of the cells. The rest of the central part of the nerve cord is occupied by the neuropile, which is a felt-like mat of fine anastamosing fibers derived from processes of the nerve cells. The nerve cells properly occupy the ventral and lateral portions of the nerve cord. They are termed bipolar or tripolar depending on the number of filamentous extensions, each cell always having more than one process, although they may appear monopolar if the two processes of a bipolar are so close together that they cannot be distinguished even after staining. The processes are distinguished as the axon, which is long, and one or more dendrites, which branch within the nervous system. Some of the cells of this region are large, pear-shaped, and unipolar. These giant cells, or ganglion cells, link up with the giant fibers by long processes. The central portion of the nerve cord consists of a mass of fibrous material, forming felt-like masses with numerous perforations.

The cerebral ganglion, which contains no motor cells, is characterized by an external layer of small spindle-shaped ganglion cells, with a few large cells interspersed between them. The neuropile, which is very dense, is connected to the ganglion cells and contains the endings of the neural paths from the nerve cord of the main nerve endings. Scattered along the length of the main nerve rings are nerve cells, which are most numerous on the posterior ring of each segment; bipolar nerve cells occur in the nerve plexus of the buccal cavity, unipolar "ganglion cells" in the epithelium of the prostomium, and in the pharyngeal and intestinal plexuses.

The cerebral ganglion and other nervous tissues also have the capability of accumulating tri-n-butyl phosphate and other man-made, mass-produced materials. Jiang et al. (2020) exposed *Eisenia fetida* to environmentally relevant or higher concentrations of TNBP (0, 0.1, 1, and 10 mg/kg) in artificial soil for 14 days. The results indicated that tri-N-butyl phosphate accumulated in the earthworm nervous tissue, specifically the cerebral ganglions. These cerebral ganglions were capable of suffering an osmotic imbalance and $Ca2^{+}$ overload due to the presence of TNBP in their environment.

The sense organs are of two main types, photoreceptor organs and epithelial sense organs. The photoreceptor organs of lumbricids are 22–63 μm long, elongated, and conical. Those occurring at the ends of the nerves to the skin are smaller than those in the epidermis and are aggregated. One or sometimes two nerves pass into each cell, and large neural fibers also extend from the nerves into the cells. Small nerves continuous with the neural fibers extend throughout the protoplasmic contents of the cells as a neurofibrillar network. In each cell, there is an ellipsoidal or elongated rod-shaped body, the optic organelle, with an outer surface consisting of interconnecting fibrillae, the retinella, and inside, a transparent hyaline substance.

The epithelial sense organs are groups of 35–45 elongated cells, broader at their bases, with distal ends terminating in sensory hairs projecting through thin regions of the cuticle. The bases of the cells end as processes, one of which extends as a nerve fiber along the basal membrane and joins the nearest epidermal nerve. Proprioceptor cells situated in the muscle layers act as tension or stretch receptors.

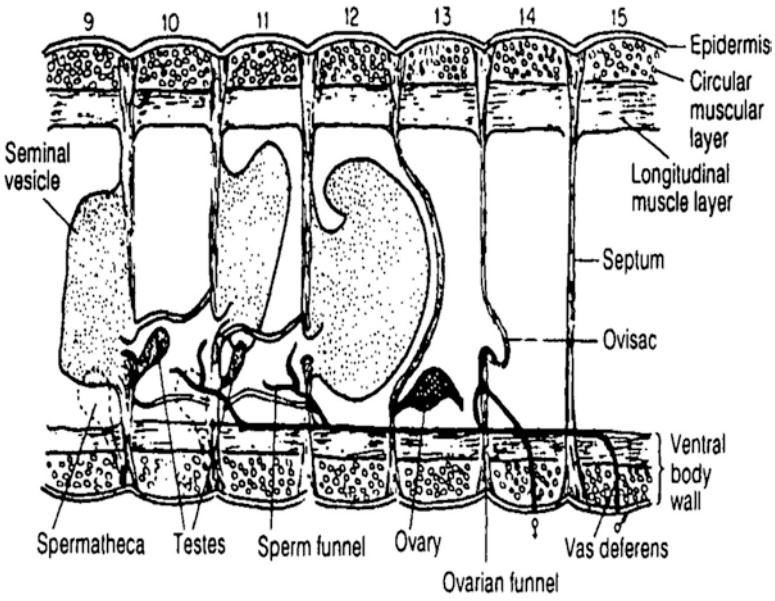

Fig. 1.14 Diagram of the reproductive system from the side

1.13 Reproductive System

Oligochaetes are hermaphrodite and have more complicated genital systems than most unisexual animals. The reproductive organs, which are confined to comparatively few segments in the anterior portion of the body (Figs. 1.7, 1.14, and 1.15), include the male and female organs and associated organs, the spermathecae, the clitellum, and other glandular structures.

The paired ovaries, which produce oocytes, are roughly pear-shaped in *Lumbricus* (or fan-shaped in *Pheretima*) and are attached by their wider ends to the ventral part of the posterior face of septum 12/13, hanging freely in segment 13, in most terrestrial species. The ovisacs are backward-facing evaginations of the anterior face of the septum immediately behind the ovaries and open into the dorsal wall of the ovarian funnels. They narrow posteriorly to form the oviducts, which in turn open onto the ventral surface of the body, their position differing between earthworm families.

The basic male organs are the testes. Most species of Lumbricidae, Megascolecidae, and Glossoscolecidae have two pairs of testes (holoandric), but some species of Lumbricidae and also the Ocnerodrilidae have only a single pair (meroandric). The testes are lobed organs attached to the posterior faces of septa 9/10 and 10/11 of *Lumbricus* and projecting from the septal walls into two median testis sacs, one in segment 10 and one in segment 11. The testis sacs are separate compartments within segments 10 and 11, lying below the ventral vessel and enclosing the ventral nerve cord. Some species, e.g., *Pheretima* spp., have much more

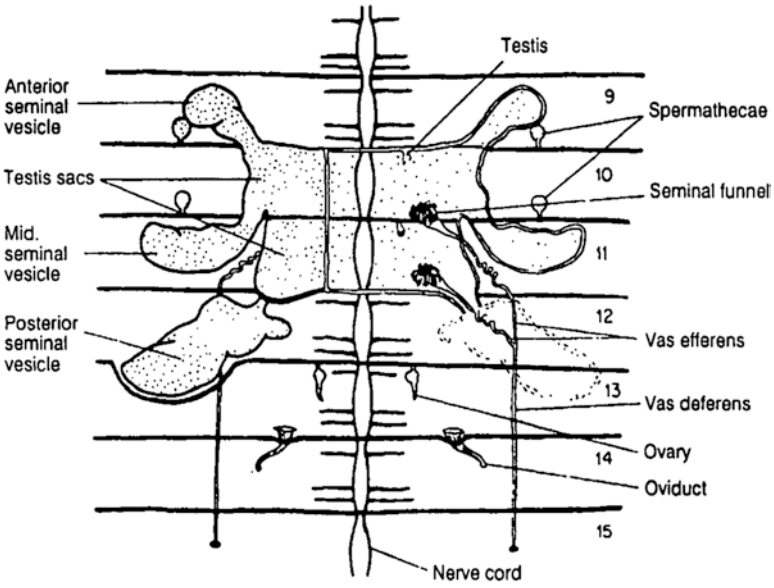

Fig. 1.15 Dissection of the reproductive system from above

extensive sacs which contain the hearts, dorsal vessel, esophagus, and seminal vesicles. These sacs are filled with nutrient fluid in which lie the developing male cells. The testis sacs communicate with the seminal vesicles (vesiculae seminalis) which are storage sacs for the developing male cells.

The seminal vesicles are the largest and most conspicuous organs of the reproductive system and are immediately obvious when earthworms are dissected as white masses on either side of the alimentary canal. They are sacs, for the most part divided by connective tissue into intercommunicating compartments. *Lumbricus* has three pairs of seminal vesicles in segments 9, 10, and 11, two pairs of which communicate anteriorly and posteriorly with the anterior testis sacs, and the third pair with the posterior testis sac in the segment immediately in front. The Megascolecidae have from one to four pairs of seminal vesicles, *Pheretima* spp. having two pairs. Seminal vesicles are never in the same segment as the testes or the testis sacs. The seminal vesicles differ in size and are sometimes very large, extending backward from the testis sacs through a number of segments, for example, they extend back as far as segment 20 in *Lumbricus castaneus*.

The sperm or funnel sacs (Fig. 1.14) are set into the posterior walls of the segments that contain them by their narrow portions. These sperm funnels are much folded, ciliated on their inner surfaces, and open into coiled or straight male ducts, the vasa efferentia. The anterior and posterior vasa efferentia on each side pass backward and join to become a vas deferens, leading to the ventral exterior surface. Some species, e.g., *Pheretima posthuma*, have the ducts associated very closely, although not actually united, and each vas deferens opens to the exterior as a male pore.

The prostates or prostatic glands are large glands associated with the posterior ends of the vasa deferentia. Their function is to produce a fluid in which sperm cells can be transferred between earthworms during copulation. In the Megascolecidae, they are of two types, tubular, or racemose (as in *Pheretima)* where the glandular tissue is compact with branching canals opening into the associated ducts. Other families have prostates in the form of muscular finger-like processes, or convoluted tubes. Prostate glands are rare in the family Lumbricidae.

Most oligochaetes have spermathecae, which are almost always paired organs: *L. terrestris* has two pairs, situated in segments 9 and 10, but other species may have more, up to a maximum of seven pairs, as in *Bimastos*, and some fewer than this or even none. The spermathecae are attached to the body wall by short stalk-like ducts. Many species of megascolecids have one or more diverticula from the spermathecal ducts.

The clitellum consists of thickened glandular epidermis, particularly on its dorsal and lateral portions. A section through the clitellum shows gland cells in three layers; those nearest the surface are mucous cells, which are similar to the mucus-secreting goblet cells in the ordinary epidermis. Also reaching the surface, but extending deeper into the clitellar tissue, are long, slender, and often convoluted cells, containing large granules. The bulk of the clitellar tissue in the deeper layer down to the basal membrane is made up of albumen-secreting gland cells, terminating distally in long slender ducts which open on to the body surface. Epidermal columnar cells also occur. The posterior segments of the clitellum are less glandular than the anterior segments, with no cocoon-secreting and albumen-secreting cells, and fewer mucous cells.

This system of reproduction can also be negatively affected by glyphosate-based herbicides that can decrease cocoon production between 69 $\pm$ 7.86% and 83 $\pm$ 10.53% (García-Pérez et al. in 2020).

References

Da Cruz Hung, I. E., Assmann, C. E., Mastella, M. H., Barbisan, F., Spillari Ruaro, R. A., Roggia, I., Turra, B. O., Chitolina, B., de Olveira Alves, A., Teixeira, C. F., Azzolin, V. F., Ribeiro, E. E., Frescura Duarte, M. M. M., & da Cruz, I. B. M. (2021). Superoxide-anion triggers impairments of immune efficiency and stress response behaviors of *Eisenia fetida* earthworms. *Chemosphere, 269*, 128712.

El-Duweini, A. K. (1965). Studies on the anatomy of *Pheretima californica*. *Bulletin of the Zoological Society of Egypt, 20*, 11–30.

García-Peérez, J. A., Alarcón-Gutiérrez, E., & Díaz-Fleischer, F. (2020). Interactive effect of glyphosate-based herbicides and organic soil layer thickness on growth and reproduction of the tropical earthworm *Pontoscolex corethrurus*. *Applied Soil Ecology, 155*, 103648.

Gardiner, M. S. (1972). *Biology of the Invertebrates*. McGraw Hill.

Hess, W. N. (1925). Nervous system of the earthworm, *Lumbricus terrestris*. *Morphology, 40*, 235–260.

Grove, A. J., & Newell, G. E. (1962). *Animal biology*. University Tutorial Press, London.

Huang, B., Long, J., Li, J., & Ai, Y. (2021). Effects of antimony contamination on bioaccumulation and gut bacterial community of earthworm *Eisenia fetida*. *Journal of Hazardous Materials, 416*, 126110.

Jiang, X., Yang, Y., Liu, P., & Li, M. (2020). Transcirptomics and metabolomics reveal Ca2+ overload and osmotic imbalance-induced neurotoxicity in earthworms (*Eisenia fetida*) under tri-n-butyl phosphate exposure. *Science of The Total Environment, 748*, 142169.

Shi, Z., Zhang, G., & Wang, C. (2018). Adsorption of phenanthrene by earthworms – A pathway for understanding the fate of hydrophobic organic contaminants in soil-earthworm systems. *Journal of Environmental Management, 212*, 115–120.

Stephenson, J. (1930). *The Oligochaeta*. Oxford University Press.

Tang, R., Lan, P., Ding, C., Wang, J., Zhang, T., & Wang, X. (2020). A new perspective on the toxicity of arsenic-contaminated soil: Tandem mass tag proteomics and metabolomics in earthworms. *Journal of Hazardous Materials, 398*, 122825.

Vail, V. A. (1972). *Contributions to North American earthworms. Natural history and reproduction in Diplocardia mississippiensis* (Bulletin Tall Timbers Research Station, 11) (pp. 1–39). Tall Timbers Research Station.

Wallwork, J. A. (1983). *Earthworm biology* (Studies in Biology No. 161). Institute of Biology, Camelot Press.

Wang, Y., Wu, Y., Cavanagh, J., Wang, X., Qiu, J., & Li, Y. (2021). Behavior and respiration responses of the earthworm *Eisenia fetida* to soil arsenite pollution. *Pedosphere, 31*, 452–459.

Xu, X., Yang, Z., Zhu, T., & Shu, W. (2021). Toxicity of soil antimony to earthowrm *Eisenia fetida* (Savingy) before and after the aging process. *Ecotoxicology and Environmental Safety, 207*, 111278.

Zhang, Y., Atopkin, D., Wang, L., & Donghui, W. (2021a). Description of a new earthworm species of the genus *Drawida* (Oligochaeta: Moniligastridae) from Northeast China and Far East Russia. *Journal of Asia-Pacific Biodiversity, 14*, 425–429.

Zhang, F., He, M., Zhang, C., Lin, D., & Zhang, J. (2021b). Combined toxic effects of dioxin-like PCB77 with Fe-based nanoparticles in earthworm *Eisenia fetida*. *Science of The Total Environment, 766*, 144347.